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Ready Biodegradability of Syntroleum Diesel Fuel S-2 by the Closed Bottle Test (OECD 301D)

Prepared for:

Syntroleum Corporation
1100, 1350 South Boulder
Tulsa, OK, USA
74119

Prepared by:

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December 17, 2001

Quality Assurance Compliance Statement

The study conducted on the ready biodegradability of Syntroleum Diesel Fuel S-2 is documented in this report. The report accurately presents the biological and chemical procedures employed in the conduct of this study and the raw data generated by those procedures (appended). The study complied with the following method:

- Ready Biodegradability: Closed Bottle Test (Guideline 301 D). Organisation for Economic Co-operation and Development, Paris 1993.

This study was conducted in accordance to the Principles of Good Laboratory Practice and Compliance Monitoring as defined by the Organisation for Economic Co-operation and Development (1998). The criterion for ready biodegradability was an oxygen demand greater than 60 % of the theoretical oxygen demand for the compound over a 14-d period in a 28-d test.

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2002/01/16
Date

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2002/01/15
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Summary

Sponsor (source):	Syntroleum Corporation 1100, 1350 South Boulder Tulsa, OK, USA 74119
Contact:	Bob Freerks 918-592-7900 tel 918-592-7979 fax
Study Director:	J. Jeffrey Wilson, Ph.D., P.Biol.
Study Location:	HydroQual Laboratories Ltd. #3, 6125 - 12 Street S.E. Calgary, Alberta, Canada T2H 2K1 Tel (403) 253-7121 / Fax: (403) 252-9363
Reference Information:	Client, 2001117; Project, 20011446; Sample, 20011446; Test, 20013619
Test Substance:	Syntroleum Diesel Fuel S-2 (RPU 10920)
Test Concentration:	1.7 mg/L
Test Dates:	November 16, 2001 to December 12, 2001
Results:	Not readily biodegradable (less than 60% degradation occurred over the 28-d test period)

Test Method

The test method complied with the Ready Biodegradability: Closed Bottle Test (Guideline 301D; Organisation for Economic Co-operation and Development, 1993). The test was conducted in a mineral medium (Table 2) inoculated with a commercial bacterial preparation. The solutions were incubated in 300 mL Biological Oxygen Demand (BOD) bottles at 20 ± 1 °C for 28 days. Oxygen levels in duplicate bottles were measured at 0, 7, 14, 21, and 28 days. Biodegradation of the test substance was assessed by the consumption of oxygen in the test bottles corrected for uptake in bottles with only inoculated medium (test control).

There were four treatments (test control, procedure control, toxicity control, and the test substance; Table 3). The test control contained inoculated mineral medium. The procedure control contained the inoculated mineral medium plus a reference material (sodium acetate). The procedure control provides information on the health of the inoculum and integrity of the test. The toxicity control contained the inoculated mineral medium, the reference material, plus the test substance. This control measured potential adverse effects of the test substance on the bacterial seed that could slow or prevent biodegradation.

The concentration of test substance was sufficient to yield an oxygen demand of 5 mg/L upon complete degradation. This value was obtained from the theoretical oxygen demand ($ThOD_{NO3}$ in mg oxygen / mg chemical) derived from the elemental composition of the substance as follows (Organisation for Economic Co-operation and Development, 1993):

$$ThOD_{NO3} = (16 * (2*c + ½ * (h - cl) + 5/2 * n + 3 *s + 5/2 * p + ½ *na - o)) / MWT$$

Where MWT is the molecular weight (g/mole) and c, h, cl, na, o, p, and s are obtained from the elemental composition:

$C_c H_h Cl_{cl} N_n Na_{na} O_o P_p S_s$

The $ThOD_{NO3}$ for Syntroleum diesel fuel S-2 is based on it being primarily a mixture of saturated C_{16} alkanes of the generic formula C_nH_{n+2} (i.e., $C_{16}H_{34}$), which is 3.46 mg molecular oxygen (O) per mg of chemical. No correction was made for nitrification because diesel fuel does not contain nitrogen. The amount of S-2 diesel fuel required to obtain a change in dissolved oxygen of 5.5 mg/L in a 300 mL BOD bottle upon complete degradation is 0.5 mg (i.e., 5.0 mg O_2 / L * 0.3 L * 2 [to convert molecular oxygen (O) to dissolved oxygen, O_2] divided by ThOD of 3.46 mg oxygen / mg S-2 diesel fuel).

The inoculum was a commercially available bacterial seed (Polyseed, InterBio, The Woodlands, Texas). One capsule was added to 500 mL of mineral medium and aerated for 2 hours. The solution was allowed to settle for 30 minutes and 100 mL removed and added to 12 L of mineral media.

Levels of dissolved oxygen were measured with a YSI Model 5760 self stirring BOD probe connected to a YSI Model 58 meter. The probe was equipped with a temperature sensor and the temperature of the test solution was recorded. The temperature of the test area was recorded daily.

The biological oxygen demand (BOD) of the test substance after each time interval was obtained by subtracting the average oxygen demand of the inoculum blank from the average value obtained from duplicate bottles for the test substance. This value was divided by the initial nominal concentration of test compound to obtain the amount of oxygen consumed per weight of test substance (mg O_2 / L ÷ (mg test compound/ L)). The percent biodegradation was calculated from the BOD exhibited by the test substance divided by the ThOD times one hundred.

Results

The Syntroleum diesel fuel S-2 was not readily biodegradable. The test substance did not meet the criterion of greater than 60 % of the $ThOD_{NO3}$ within a 14-d window over the 28-d test period. The amount of biodegradation of the test substance at 28 days was 53%.

Protocol Deviations

There were no protocol deviations during the conduct of this study. Oxygen depletion in the inoculum blank did not exceed 1.5 mg per liter after 28 days of incubation. The variance amongst duplicate bottles was less than 20 %. Biodegradation of the sodium acetate reference compound met the criterion of 60 % of the $ThOD_{NO3}$ within 14 days. The test substance did not inhibit degradation of the reference compound by more than 25 % after 14 days.

2. References

Organisation for Economic Co-operation and Development, 1993. Ready Biodegradability: Closed Bottle Test (Guideline 301D); OECD, Paris.

Organisation for Economic Co-operation and Development, 1998. Principles of Good Laboratory Practice and Compliance Monitoring, OECD, Paris.

Table 1. Summary of Test Conditions

Parameter	Test Condition
Test type	Static
Duration	28 days
Inoculum	Polyseed
Temperature	20 ± 1 °C
DO determination method	Electrode
Initial dissolved oxygen	8.1 mg/L
Test vessel	BOD bottles
Test volume	300 mL
Replicates	Two at each of five time intervals
Aeration	None
Controls	1. Test (inoculum blank) 2. Procedure (reference compound plus inoculum) 3. Toxicity (reference compound, test substance and inoculum)
Concentration of test compound	1.7 mg/L
Concentration of Reference Substance (Sodium Acetate)	7.0 mg/L
Criterion for Ready Biodegradability	60% ThOD in 14-d window within 28 days

Table 2. Test Results Summary

Treatment	Criterion	Result
Test Control	Change in dissolved oxygen over 28 days must < 1.5 mg/L	Pass
Procedure Control	Degradation of reference substance must be >60% of $ThOD_{NO3}$ within 14 days	Pass
Toxicity Control	Toxic if degree of inhibition is >25% of procedure control within 14 days	Pass - not toxic
Test Substance	Biodegradable if oxygen consumption exceeds 60% of $ThOD_{NO3}$ within a 14 day window	Not readily biodegradable

Table 3. Summary of Dissolved Oxygen Determinations

Bottle Contents	Calculation Data (used in Table 4)	Dissolved Oxygen (mg O_2/L after n days)				
		0	7	14	21	28
Test control	c_1	7.8	6.4	6.2	6.5	6.5
	c_2	7.8	6.1	6.0	na[1]	6.7
	$m_b = (c_1 + c_2)/2$	7.8	6.3	6.1	6.5	6.6
Test substance	a_1	8.0	5.9	5.6	4.6	3.3
	a_2	8.0	4.8	5.4	4.2	3.8
	a_3					3.5
Procedure control	r_1	8.0	5.9	2.8	2.0	2.1
	r_2	8.0	3.2	2.5	1.8	2.0
Toxicity control	tox_1	8.0	3.1	1.5	1.6	1.2
	tox_2					1.1

1 – Day 21 duplicate control sample lost before measurement

Table 4. Percent Degradation Calculations (symbols defined in Table 5)

Calculations (as per OECD 301D Method)	Test Day 7	14	21	28
$(m_{b(0)}.m_{b(28)})$ < 1.5 mg/L				1.2
Inoculum Control Validity Criterion				**PASS**
Test Substance (Syntroleum Diesel Fuel S-2)				
$(m_b\text{-}a_1)$	0.4	0.5	1.9	3.3
$(m_b\text{-}a_2)$	1.5	0.7	2.3	2.8
$(m_b\text{-}a_3)$				3.1
% Da_1 = 100 x $(m_b\text{-}a_1)$ / (Diesel S-2 mg/L x ThOD)	6	9	33	57
% Da_2 = 100 x $(m_b\text{-}a_2)$ / (Diesel S-2 mg/L x ThOD)	25	12	39	48
% D_{mean} = $(Da_1 + Da_2)/2$	15	10	36	53
Ready Biodegradable (%D_{mean} >60%?) (YES or NO)				**NO**
Check Final pH (>6.0)				7.3
Reference Substance (Sodium Acetate)				
$(m_b\text{-}r_1)$	0.4	3.3	4.5	4.5
$(m_b\text{-}r_2)$	3.1	3.6	4.7	4.6
% Dr_1 = 100 x $(m_b\text{-}r_1)$ / (Sodium acetate mg/L x ThOD)	6	60	82	82
% Dr_2 = 100 x $(m_b\text{-}r_2)$ / (Sodium acetate mg/L x ThOD)	56	66	86	84
% Dr_{mean} = $(Dr_1 + Dr_2)/2$	31	**63**	84	83
% Dr_{mean} must be ≥60% within 14 days		**PASS**		
Toxicity Check				
$(m_b\text{-}tox_1)$	3.2	4.6	4.9	5.4
% Dr_1 = 100 x $(m_b\text{-}r_1)$ / (Sodium acetate mg/L x ThOD)	58	84	90	99
% $D_{inhibition}$ = 100 x (%Dr_{mean} - % $Dtox_1$)/% Dr_{mean}	-85	**-33**	-7	-19
Toxic if % $D_{inhibition}$ >25% within 14 days		**PASS**		